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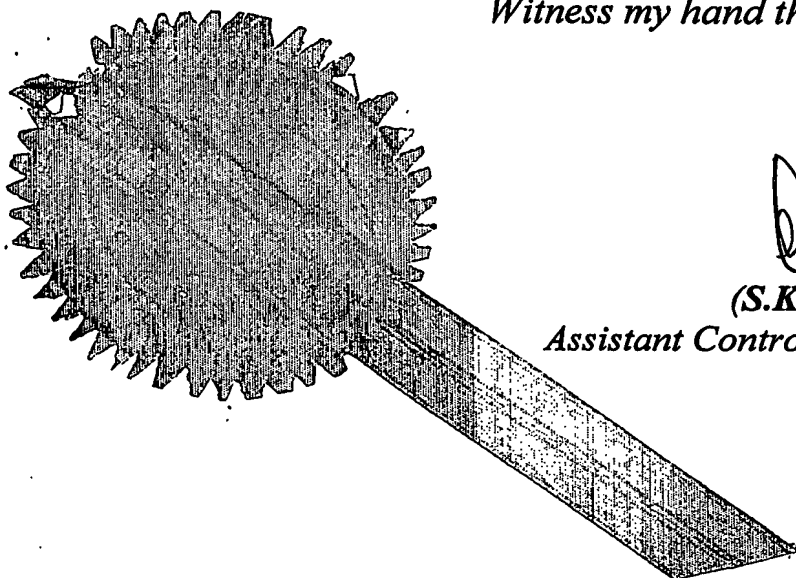
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IN/04/206

*I, the undersigned being an officer duly  
authorized in accordance with the provision of the  
Patent Act, 1970 hereby certify that annexed hereto is  
the true copy of the Application and Complete  
Specification filed in connection with Application for  
Patent No.892/Del/2003 dated 14<sup>th</sup> July 2003.*

*Witness my hand this 9<sup>th</sup> day of August 2004.*



(S.K. PANGASA)

Assistant Controller of Patents & Designs

0892 DEL 03

14 JUL 2003

**FORM 1**  
**THE PATENTS ACT, 1970**  
**(39 of 1970)**  
**APPLICATION FOR GRANT OF A PATENT**  
**(See sections 5(2), 7, 54 and 135 and rule 39)**

1. I/We, The Energy and Resources Institute, an *Indian registered body incorporated under the Registration of Societies Act (Act XXI of 1860)*, Darbari Seth Block, Habitat Centre, Lodhi Road, New Delhi 110 003, India and Institute of Reservoir Studies, Oil and Natural Gas Corporation, an Indian company registered under Company Act 1956 having its registered office at Jeevan Bharati, Connaught Place, New Delhi 110001, India
2. hereby declare -
  - (a) that I am/we are in possession of an invention titled: **A process for enhanced recovery of crude oil from oil wells using novel multi-microbial strain.**
  - (b) that the Provisional/Complete specification relating to this invention is filed with this application.
  - (c) that there is no lawful ground of objection to the grant of a patent to me/us.
3. further declare that the inventor(s) for the said invention is / are :
  - (a) LAL, Banwarl
  - (b) REDDY, .Mula Ramanjaneya Varaprasada  
all of The Energy and Resources Institute, Darbari Seth Block,  
Habitat Centre, Lodhi Road, New Delhi 110 003
  - (c) AGNIHOTRI, Anil
  - (d) KUMAR. Ashok,
  - (e) SARBHAI, Munish Prasad,
  - (f) SINGH, Nimmi,
  - (g) KHURANA, Raj Karan, and
  - (h) KHAZANCHI, Shibben Kishen  
all of Institute of Reservoir Studies, Oil and Natural Gas  
Corporation, Chandekhedha Campus, Ahmedabad; and  
all are Indian citizen
4. I/We, claim the priority from the application(s) filed in convention countries, particulars of which are as follows: NIL

DUPLICATE

5. I./We, state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which I/We are the applicant/patentee : NA
6. I/We, state that the application is divided out of my/our application, the particulars of which are given below: Nil  
Application No.: Nil and pray that this application deemed to have been filed on .....NA..... under section 16 of the Act.
7. That I am/we are the assignee or legal representative of the true and first inventors.
8. That my/our address for service in India is as follows:

**LAKSHMIKUMARAN & SRIDHARAN**  
B4/158, SAFDARJUNG ENCLAVE,  
NEW DELHI 110 029, INDIA  
Tel: 011- 2619 2243/73/80 Fax: 2619 7578

9. Following declaration was given by the inventor(s) or applicant(s) in the convention country:  
  
I/We the true and first inventors for this invention of or the applicant(s) in the convention country declare that the applicant(s) herein is/are my/our assignee or legal representative.
10. That to the best of my /our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to me/us on this application
11. Following are the attachments with the application:
- (a) Complete specification (3 copies) / with 1 copy in electronic format as a Floppy diskette.
  - (b) Drawings Nil
  - (c) Priority document - NIL
  - (d) Statement and undertaking on FORM-3
  - (e) Power of Attorney – to follow
  - (f) Form 5
  - (g) Official fee Rs. 3000/- in Cash / Cheque/ Bank Draft bearing No.....date .....on.....Bank

I/We request that a patent may be granted to me/us for the said invention.

Dated this day of 14 July, 2003



**V. Lakshmikumaran**  
**Of LAKSHMIKUMARAN & SRIDHARAN**  
**Attorney for the applicants**

To  
The Controller of Patents  
The Patent Office, at Delhi.



0092-03  
FORM 2

THE PATENTS ACT, 1970  
(39 of 1970)

14 JUL 2003

PROVISIONAL / COMPLETE SPECIFICATION  
(See section 10)

**A process for enhanced recovery of crude oil  
from oil wells using novel multi-microbial  
strain**

DUPLICATE

**THE ENERGY AND RESOURCES INSTITUTE** an Indian registered body  
incorporated under the Registration of Societies Act (Act XXI of 1860), Darbari Seth  
Block, Habitat Centre, Lodhi Road, New Delhi 110 003, India

And

**INSTITUTE OF RESERVOIR STUDIES, Oil and Natural Gas  
Corporation**, an Indian Company registered under Company Act 1956, having its  
registered office at Jeevan Bharti, Connaught Place, New Delhi

The following specification particularly describes the nature of the invention and the manner in  
which it is to be performed.

## **A process for enhanced recovery of crude oil from oil wells using novel multi-microbial strain**

### **Field of the invention**

The present invention relates to a process of enhancing the recovery of crude oil from oil wells using novel microbial consortium, proliferated in novel nutrient mediums I and II. The said consortium has been deposited with the Institute of Microbial Technology, Chandigarh having accession no MTCC No.S2-001. The said micro-organism consortium has been isolated from a sample of formation water of an oil-well located at Sobhasan, Mehasana Gujarat, India.

The present invention particularly relates to the use of a multi-strain microbes having anaerobic, barophilic, hyper thermophilic property, *in situ* application hither to called MMMAP (Multi-strain Mixed Microbial Application), for enhancement of crude oil recovery from oil wells. The invention also relates to production of volatile fatty acids leading to alteration in rock characteristics thereby enhancing the oil recovery from the oil wells.

### **Background and prior art reference**

There is a strong relationship between continued industrialization coupled with economic growth and an increase in the demand for oil, especially for the fuel. The demand for crude oil has exceeded the existing production in India and over and above has been demanding more imports, thereby increasing the reliance on those countries that supply oil. The existing conventional oil production technologies are able to recover only about one-third of the oil originally in place in a reservoir. New technologies for recovering this residual oil offers the most timely and cost effective solution to reverse the decline in domestic oil production and to increase the oil reserves of the state. Microbial based oil recovery process is one of such methodologies and has several unique advantages that make it an economically attractive alternative to other processes for enhanced oil recovery. This process does not consume large amounts of energy, as do thermal recovery processes and they do not depend on the price of crude oil, as is the case with many chemical recovery processes. Because microbial growth occurs at exponential rates, it should be possible to produce large amounts of useful products quickly from inexpensive and renewable resources. Economic analysis of some MEOR (Microbial

Enhanced Oil Recovery) field trials showed that MEOR based oil recovery has produced oil for as little as three dollars per barrel [Knapp et al., 1992; Bryant et al.1993],

5 The MEOR processes can be categorized into three main domains depending on the type of production problem and where the process occurs in the reservoir [Jenneman, 1998]. The much talked-about well bore clean out processes involve the use of hydrocarbon-degrading or scale-removing bacteria to remove deposits from tubing, rods, and other surfaces in the well and thereby avoiding frequent chemical treatments to maintain oil production. It greatly reduces operating costs and extends the lifetime of the well [Raiders et al. 1989]. This approach is a mature commercial technology with thousands of wells treated on a regular basis [McInerney et al.1985; Nelson and Schneider, 1993]. The next MEOR technology is well stimulation where an oil well close to its economic limit is treated with a mixture of anaerobic bacteria and a fermentable carbohydrate, usually molasses [Hitzman, 1983]. The production of acids, solvents, and gases in the well bore region is believed to alter the oil/rock characteristics and improve the drainage of oil into the well.

Microbial enhanced water flooding processes are done late in the course of a water flood and involve the injection of nutrients and or microorganisms into the reservoir in order to stimulate microbial activity throughout the reservoir. In carbonate formations, the production of organic acids by the microbial fermentation of carbohydrates is believed to alter pore structure due to the dissolution of the carbonate minerals and substantial improvements in oil production have been reported with this process [Knapp et al., 1992; Wagner et al., 1995]. In sandstone formations, substantial increase in oil production require that the interfacial tension between the oil and water phases be reduced by a factor of 10,000 or more in order to release the oil that is entrapped in small pores by capillary pressure. The lipopeptide biosurfactant produced by *Bacillus licheniformis* strain JF-2 substantially reduces the interfacial tension between oil and water [Lazar et al., 1993; Lin et al., 1994]. The introduction of this organism along with other anaerobic bacteria in two field trials in Oklahoma has increased oil production and decreased the water to oil ratio of the produced fluids [Bryant et al., 1993]. The addition of nitrate and/or inhibitors of sulfate reduction to injection waters are also used to control hydrogen sulfide production and improve oil recovery [Telang et al., 1997; Streeb and Brown, 1992].



In addition to the above approaches, a microbial plugging process to reduce permeability variation in oil reservoirs in order to improve the performance of water flood was developed [McInerney et al., 1990; McInerney et al., 1999]. The injection water preferentially flows through the most permeable layers of the rock with little or no movement in the less permeable regions. The oil present in the low permeable regions is by-passed and unrecovered. The stimulation of the growth of indigenous microorganisms in the high permeability regions by nutrient injection reduces water movement in these regions and diverts fluid flow into the less permeable regions of the reservoir that have high oil saturation. Laboratory experiments have shown that in situ microbial growth substantially reduces the permeability of sandstone cores, that microbial growth occurs preferentially in the high permeability regions, and that plugging of the high permeability regions diverts fluid flow into less permeable regions [Portwood, 1995; Raiders et al., 1986]. Since the process does not require the production of a specific chemical or the growth of a specific organism, it should be applicable in many reservoirs.

MEOR methods take advantage of the ability of microbes to produce products such as gases, surfactants, acids, solvents, and polymers/ biomass for improving oil recovery. These products, in turn, can change oil/rock properties in a positive direction, and thus facilitate additional oil recovery. To be successful, microbes must be able to live and proliferate to the expected level in the harsh reservoir environment. In natural conditions the non-conductive and nutrient limiting conditions play a very important role in keeping the ecological balances of the population system. To stimulate the successions of desirable organisms there is need to modify such environments through introduction of specific and selective nutrients or to introduce the desirable populations or through suppression of non-desirable populations. In any of these cases the criteria to be followed, of course with certain exceptions, would be (1) salinity less than 15% NaCl; (2) temperature less than 180 °F; (3) depth less than 8,000 ft; (4) trace elements (As, Se, Ni, Hg) less than 10-15 ppm; (5) permeability greater than 50 md; (6) oil gravity greater than 15 ° API; and (7) residual oil saturation greater than 25%.

In the earlier MEOR methods, the microorganism(s) could not survive at a temperature beyond 70°C. The above draw back was overcome by providing a unique combination of novel micro-organisms and novel nutrient mediums. The micro-organism(s) were found to be highly active at 90°C. The medium supports the growth and

proliferation of the culture in extreme conditions. The metabolic products produced by consuming these nutrients protect the microbes. The composition of the nutrients also promotes selectivity of the bacterial growth of the present invention. In addition, the use of formation water provides appropriate concentration of salts in the nutrient medium and the absence of anaerobic bacteria which are harmful to oil reservoirs is avoided. Also, formation water used is compatible with oil reservoir and helps in the growth of multi bacterial strain of the present invention.

#### **Objects of the invention**

The main object of the present invention is to provide use of a multi-strain mixed microbial application process for improving the sweep efficiency in order to enhance oil recovery.

Another object of the present invention is to provide a multi-strain mixed microbial application process for reducing viscosity of oil by the production of gases and alteration in the rock characteristics through production of volatile fatty acids in order to increase oil recovery.

Still another object of the present invention is to provide a stimulating nutritional medium for promoting the growth of the hither to applied multi-strain mixed microbial population to proliferate and continue in the oil well after its application for longer periods.

Yet another object of the invention is to provide nutrient mediums for selective mass scale production of said population of bacterial consortium in laboratory and further proliferation of the hither to applied multi-strain population in the oil-well.

#### **Summary of the invention**

Accordingly the present invention relates to enhancement of oil recovery from the oil wells by applying a multi-bacterial strain proliferated in novel nutrient mediums. The present invention also relates to a process of enhancing the recovery of crude oil from oil wells using novel microbial consortium deposited with Institute of Microbial Technology, Chandigarh having accession-no MTCC No.S2-001. The said micro-organisms have been isolated from a sample of formation water of an oil-well located at Sobhasan, Mehasana Gujarat, India.

The present invention particularly relates to use of a multi-strains anaerobic, barophilic, hyper thermophilic, microbial *in situ* application process hither to called

MMMAP, for enhancement of crude oil recovery from oil wells. The invention also relates to production of volatile fatty acids leading to alteration in rock characteristics thereby enhancing the oil recovery from the oil wells.

## 5 Detailed description of invention

In accordance, the present invention provides a process for enhancing the oil recovery from oil well, the said process comprising steps of:

- a) dissolving mineral nutrients nitrogenous substances, reducing agents, buffering agent, carbon source and trace mineral in formation water, adjusting the pH in the range 7.0 to 7.5,
- b) autoclaving the step (a) solution at a temperature in the range of 120°-125°C, at a pressure of 15-20 psi for a time period of 20-25 minutes,
- c) maintaining the temperature of step (b) solution up to 90°C, adding vitamin solution, adjusting the pH to 6.50 to 7.50 using an alkali to obtain nutrient medium I;
- d) inoculating with multibacterial strain in step (c) nutrient medium I in the presence of anaerobic gas mixture of  $N_2$   $CO_2$ ,  $H_2$ ;
- e) incubating the step (d) mixture at 90°C;
- f) obtaining the seed population of multibacterial strain;
- g) preparing nutrient medium II by adapting steps (a) to (c);
- h) inoculating the seed multibacterial strain under aseptic condition to step (g) nutrient medium II by maintaining impeller speed in the range of 30 to 40 r.p.m. anaerobic gas purging at the rate of 0.2 -0.5vvm at a pH in the range of 7.0 to 7.50 and at a temperature in the range of 80°-90° C to obtain a biological solution;
- i) injecting the biological solution of step (h) to the oil well, followed by water to displace about 20% of entire biological solution, and
- j) obtaining the enhanced oil recovery from oil wells.

In an embodiment of the present invention provides a process, wherein the nutrient medium I used comprises of:

Mineral nutrients	Quantity/Litre
$MgSO_4 \cdot 7H_2O$	0.5 to 1.5 g
$K_2HPO_4$	0.4 to 0.6 g

$\text{KH}_2\text{PO}_4$	0.1 to 0.6 g
<b>Nitrogenous substrates</b>	
$\text{NH}_4\text{Cl}$	0.5 to 1.5 g
Yeast extract	1.0 to 4.0 g
Tryptone	0.5 to 1.0 g
<b>Reducing agents</b>	
Cystein HCL	1.0 to 5.0 g
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	2.0 to 5.0
<b>Carbon Source</b>	
Molasses	50.0 to 100 g
Corn steep liquor	50.0 to 100 g
<b>Buffering agent</b>	
$\text{NaHCO}_3$	1.0 to 2.5 g
Vitamin stock solution	10 to 20 ml
Trace mineral stock solution	15 to 20 ml

In another embodiment of the present invention provides a process, wherein the nutrient medium II used comprises of:

Mineral nutrients	Quantity/Litre
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.05 to 0.15 g
$\text{K}_2\text{HPO}_4$	0.2 to 0.4 g
$\text{KH}_2\text{PO}_4$	0.2 to 0.4 g
<b>Nitrogenous substrates</b>	
$\text{NH}_4\text{Cl}$	0.5 to 1.5 g
<b>Reducing agents</b>	
Cystein HCL	0.1 to 0.5 g
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0.1 to 0.5 g
<b>Carbon Source</b>	
Molasses	15.0 to 30.0
Corn steep liquor	50.0 to 100 g
<b>Buffering agent</b>	
$\text{NaHCO}_3$	1.0 to 2.5 g
Vitamin stock solution	5 to 10 ml
Trace mineral stock solution	5 to 10 ml

5

Another embodiment of the invention, the trace mineral solution in formation water having a pH in the range of 6.50 to 7.50 of nutrient medium I, comprises of:-

Chemicals	Quantity per litre
Nitrilotriacetic acid (sodium salt)	0.82 to 2.00 g
MgSO <sub>4</sub>	2.5 to 3.2 g
MnSO <sub>4</sub> .2H <sub>2</sub> O	0.2 to 0.8 g
NaCl	0.7 to 1.2 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.05 to 0.12 g
CoCl <sub>2</sub> /CoSO <sub>4</sub>	0.07 to 0.14 g
ZnSO <sub>4</sub>	0.08 to 0.12 g
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.008 to 0.015 g
AlK (SO <sub>4</sub> ) <sub>2</sub>	0.007 to 0.015 g
H <sub>3</sub> BO <sub>3</sub>	0.009 to 0.012 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.008 to 0.13 g

Still another embodiment of the invention provides a process, wherein the vitamin solution in distilled water having a pH in the range of 6.50 to 7.50 of nutrient medium I, comprises of:

5

Vitamin	Quantity mg/l
Biotin	1.2 to 2.2
Folic acid	1.7 to 2.4
Pyridoxine HCl	7.0 to 12.0
Thamine HCl	7.0 to 12
Riboflavin	6.0 to 7.0
Nicotinic acid	4.0 to 6.0
DL-Calcium Pantothenate	3.0 to 6.2
P-Aminobenzoic acid	4.1 to 5.6
Vitaminin B <sub>12</sub>	0.08 to 13.0
Lipoic acid	2.8 to 5.4

Yet another embodiment of the invention provides a process, wherein the trace mineral solution in formation water having a pH in the range of 6.50 to 7.50 of nutrient medium II, comprises of:

10

Chemicals	Quantity per litre
Nitrilotriacetic acid (sodium salt)	0.82 to 2.00 g
MgSO <sub>4</sub>	2.5 to 3.2 g
MnSO <sub>4</sub> .2H <sub>2</sub> O	0.2 to 0.8 g
NaCl	0.7 to 1.2 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.05 to 0.12 g

CoCl <sub>2</sub> /CoSO <sub>4</sub>	0.07 to 0.14 g
ZnSO <sub>4</sub>	0.08 to 0.12 g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.008 to 0.015 g
AlK (SO <sub>4</sub> ) <sub>2</sub>	0.007 to 0.015 g
H <sub>3</sub> BO <sub>3</sub>	0.009 to 0.012 g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.008 to 0.13 g

Yet another embodiment of the invention provides a process, wherein the vitamin solution in distilled water having a pH in the range of 6.50 to 7.50 of nutrient medium II, comprises of:

5

Vitamin	Quantity mg/l
Biotin	1.2 to 2.2
Folic acid	1.7 to 2.4
Pyridoxine HCl	7.0 to 12.0
Thamine HCl	7.0 to 12
Riboflavin	6.0 to 7.0
Nicotinic acid	4.0 to 6.0
DL-Calcium Pantothenate	3.0 to 6.2
P-Aminobenzoic acid	4.1 to 5.6
Vitamin B <sub>12</sub>	0.08 to 13.0
Lipoic acid	2.8 to 5.4

Yet another embodiment of the invention, the alkali used is selected from a group consisting of sodium hydroxide and potassium hydroxide.

Yet another embodiment of the invention, the nutrient mediums I and II are suitable for growing multibacterial strain consisting of *Thermoanaerobacterium sp.*, *Thermotoga sp.* and *Thermococcus sp.*

10

Yet another embodiment of the invention, the multibacterial strain obtained possesses barophylic, thermophilic and anaerobic properties.

Yet another embodiment of the present invention, the nutrient medium II provides growth and proliferation of multi bacterial strain up to a temperature of 90°C in the oil well thereby enhancing the oil recovery.

15

Yet another embodiment of the invention, the nutrient medium II helps in the growth of multi bacterial strain leading to the production of volatile fatty acids and carbon dioxide in the oil wells to enhance the recovery of oil.

Yet another embodiment of the invention, the volatile fatty acid produced, reduces  
5 the viscosity of the oil in the oil well.

In yet another embodiment of the invention provides a process, wherein the carbon-dioxide produced helps in sweeping the oil from the oil well.

Still another embodiment of the invention provides a process, wherein the oil recovery is enhanced up to 3 folds.

10 All the chemicals are mixed in RO (reverse osmosis) water to 1000 ml with pH adjustment to 7.0- 7.5 with 1 N NaOH, and autoclave nutrient mixture at the temperature of around  $121 \pm 5^{\circ}\text{C}$  at the pressure of 15 – 20 psi for a period of 20-25 minutes, cooling the said mixture containing medium to the well temperature before inoculating the multi-strain mixed microbial population for enrichment.

15 The mixture of nutrient medium is prepared, by mixing all the components mentioned above except the reducing agents and vitamins in a 2 litre glass flask. The medium in the glass flask is heated to  $90^{\circ}\text{C}$  in water bath with a temperature regulator set at  $90^{\circ}\text{C}$ . The medium is purged with anaerobic gas mixture comprising of  $\text{N}_2$ ;  $\text{CO}_2$ ;  $\text{H}_2$  in a  
20 ratio of 80: 10: 10 and the reducing agents are added. Then medium is dispensed into specialized anaerobic vessels using 50 ml disposable syringe. The vessels are then properly plugged and sealed with rubber stopper and aluminum crimps. Then vessels are autoclaved at  $121 \pm 5^{\circ}\text{C}$  and 15-20 psi pressure for 20-25 minutes. The vessels are removed from the autoclave and transferred immediately to a water bath maintained at  
25  $90^{\circ}\text{C}$ . The vitamin solution is added to the autoclaved nutrient mixture medium and the multi-strain mixed microbial population is inoculated into this medium. The overhead space of the vessels is filled with the anaerobic gas mixture of  $\text{N}_2$ ;  $\text{CO}_2$ ;  $\text{H}_2$  in a ratio of 80: 10: 10. The inoculated vessels are then incubated at  $90^{\circ}\text{C}$ .

30 According to this invention, the mass culturing of the multi-strain mixed microbial population is done in a specially designed anaerobic bioreactor of 100 litre capacity with high temperature and pressure controls facilities. In accordance with this invention the nutrient mixture used contains all the components mentioned above and preparation

procedure as described above. The medium is prepared in the 100 litre capacity anaerobic bioreactor and autoclaved at  $121 \pm 5^\circ\text{C}$  and 15-20 psi for 20 -25 minutes. After the sterilization the anaerobic bioreactor is flushed with sterile anaerobic gas mixture comprising  $\text{N}_2$ ;  $\text{CO}_2$ ;  $\text{H}_2$  in a ratio of 80: 10: 10 and vitamin mixture is added. The impeller speed, anaerobic gas purging, pH regulation and temperature controls are set at 30 - 40 rpm 0.2-0.5 vvm 7.0 - 7.5 and  $80-90^\circ\text{C}$  respectively. The inoculum thus developed is used for MEOR field test.

The fully grown multi-strain thermophilic anaerobic culture is transferred aseptically from bioreactor to specially designed insulated containers with continuous purging of anaerobic gas mixture. The containers are sealed and transported to MMMAP application well site. Total volume of biological solution to be injected depends on the pay zone thickness, porosity, radius of treatment, injectivity etc. As pre-flush, nutrient medium in the quantity 20-30% of total biological solution is pumped into the well. Then 1-2 % multi strain anaerobic thermophilic culture is added to nutrient medium, blended and pumped into the well, followed by an after flush (nutrient medium only) of about 20% of biological solution. Then a volume of water is injected adequate to displace the entire biological solution through the perforations and into the target reservoir.

The nutrient medium of the present invention provides salts like sodium chloride help in increased salt-bridges in the proteins.  $\text{H}_2$ ,  $\text{S}_2$ ,  $\text{S}_2\text{O}_3$  serve as electron donors and  $\text{Na}_3$  ions as electron acceptor. The nitrogenous material helps in the protein synthesis which is important for increased production of induced proteins at higher temperature. The adaptability is achieved by  $\text{K}^+$  ions, amino acids produced from the nutrients supplied and sugars (which is present at a level of 40% of the total sugars in molasses). Iron, copper, manganese, tungsten etc are used as common enzyme ingredients.

A multi-strain mixed microbial process for microbial enhanced oil recovery from oil wells by reducing viscosity of oil and by increase in sweep efficiency through production of volatile fatty acids and carbon dioxide gas is thus invented and preparation of the process thereof according to a preferred embodiment is herein described in the following example"

#### Novelty

The novelty of the present invention is to stabilize bacterial activity of multi bacterial strain at a high temperature up to  $90^\circ\text{C}$  using novel composition of a nutrient



media The media supports the growth and proliferation of multibacterial stain both in laboratory and oil well under extreme conditions. The metabolic products produced by consuming these nutrients protect the microbes. The composition of the nutrients also promotes selectivity of the bacterial growth of the present invention.

In addition, the use of formation water provides an appropriate concentration of salts in the nutrient medium and the absence of anaerobic bacteria which are harmful to oil reservoirs is avoided. Also, formation water used is compatible with oil reservoir and helps in the growth of multi bacterial strain of the present invention.

The following examples illustrate the invention which should not be construed to limit the scope of the present invention.

### Examples

#### Example 1

Microbial nutrient mixture, containing NaCl 0.01 – 0.02 %  $MgCl_2 \cdot 7 H_2O$ ; 0.04 – 0.06%  $K_2HPO_4$ ; 0.4 – 0.6%  $KH_2PO_4$ ; 0.1 - 0.2 Resazurin; 0.1% Cysteine HCl; 0.07 %  $Na_2S \cdot 9H_2O$ ; 0.1-0.5%  $NaHCO_3$  (added separately from a stock of 2%) ; 0.2-0.5% Vitamin solution (v/v); 1 – 2% Trace mineral solution; 1-2% Molasses and Corn steep liquor mix; 1.5 - 2% oil well formation water; 50-60 % and then mixed in RO (reverse osmosis) water to 1000 ml adjusting the pH 7.0 to 7.5 and autoclaved the mixture without adding vitamin solution at a temperature of 121°C and 15-20 psi pressure for 20-25 minutes.

The trace elements solution used for the nutrient mixture is comprising 0.05-0.12% nitrilotriacetic acid; 0.1-0.2%  $MnSO_4 \cdot 2H_2O$ ; 0.001-0.002%  $FeSO_4$ ; 0.001-0.007%  $CaCl_2$ ; 0.001-0.003%  $AlK(SO_4)_2$ ; 0.001-0.006%  $H_3BO_3$ ; 0.001-0.002%  $Na_2MoO_4 \cdot 2H_2O$  The Vitamin solution contained 0.001-0.002% biotin; 0.0001- 0.0003% folic acid; 0.01-0.03% pyridoxine-HCl; 0.0001-0.0004% riboflavin; 0.0001-0.0004% thiamine HCl; 0.001-0.0005% nicotinic acid; 0.0001-0.0005% calcium D pantothenate; 0.0001-0.0003% vitamin  $B_{12}$ ; 0.0001-0.0004% PABA; 0.0001-0.0005% lipoic acid. The pH of the mixture is adjusted to 6.8 with 1.0 N NaOH and the mixture is sterilized aseptically with 0.22 $\mu$  membrane filter assembly.

The designed nutrient mixture is dispensed hot in specialized anaerobic vessels and reduced while purging with anaerobic gas mix, by adding reducing agents. The vessels are

sealed and autoclaved. After autoclaving, the nutrient mixture is kept at 90°C in a temperature controlled hot water bath. The medium is inoculated with multi-strain mixed microbial population selected from a group consisting of *Thermoanaerobacterium sp.*, *Thermotoga sp.* and *Thermococcus sp.* to prepare the inoculum ready for scale up or mass production of the same for in situ application process MMMAP.

For the application of MMMAP in oil wells; the wells were identified on the basis of reservoir and fluid properties viz. permeability > 25 md, porosity > 20%, salinity of oil associated water up to 10%, °API of crude oil > 15 API, viscosity of oil < 30 cp, water cut (coproduced) 30-90%, reservoir temperature up to 90°C and residual oil saturation minimum > 25%.

The multi-strain thermophilic anaerobic culture is transferred aseptically from bioreactor to specially designed insulated containers with continuous purging of anaerobic gas mixture. The containers are sealed and transported to MMMAP application well site. Total volume of biological solution to be injected depends on the pay zone thickness, porosity radius of treatment, injectivity etc. As pre-flush, nutrient medium in the quantity 20-30% of total biological solution is pumped into the well. Then 1-2 % multi strain anaerobic thermophilic culture is added to nutrient medium, blended and pumped, followed by injection of after flush treatment (nutrient medium only). The total biological solution is displaced by volume of water adequate to inject the entire biological solution through the perforations and into the target reservoir. All the injections are made at surface pressure not exceeding hydro fracturing pressure of the formation.

After the application of the MMMAP in oil wells, the oil wells are closed for 20-30 days. The process MMMAP stimulated the microbial growth in situ and produced their metabolic products in particular the carbon dioxide which reduce the viscosity of oil. The end product of the application of MMMAP in oil wells is increased oil recovery up to 3 folds. The multi-strain mixed microbial metabolic products are carbon dioxide and volatile fatty acids which are harmless and which do not adversely affect the oil quality.

### Example 2

All conditions used are identical as in example 1 above, except that a combination of any two bacteria are used from the group consisting of *Thermoanaerobacterium sp.*,  
5 *Thermotoga sp.* and *Thermococcus sp.* to provide enhancement of oil recovery up to 0.8 fold.

### Example 3

All conditions used are identical as in example 1 above, except that any one  
10 bacterium is used from the group consisting of *Thermoanaerobacterium sp.*, *Thermotoga sp.* and *Thermococcus sp.*, to provide enhancement of oil recovery up to 0.3 fold.

### Advantages of the present invention

1. Provides novel nutrient mediums for the growth of the multi bacterial strain up to a  
15 temperature of 90°C.
2. The present invention provides an efficient process for the enhanced oil recovery from oil wells.

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I/We claim:

1. A process for enhancing the oil recovery from an oil well, the said process comprising steps of:

- a) dissolving mineral nutrients nitrogenous substances, reducing agents, buffering agent, carbon source and trace mineral in formation water, adjusting the pH in the range 7.0 to 7.5,
- b) autoclaving the step (a) solution at a temperature in the range of 120°-125°C, at a pressure of 15-20 psi for a time period of 20-25 minutes,
- c) maintaining the temperature of step (b) solution up to 90°C, adding vitamin solution, adjusting the pH to 6.50 to 7.50 using an alkali to obtain nutrient medium I;
- d) inoculating multibacterial strain using step (c) nutrient medium I in the presence of anaerobic gas mixture of N<sub>2</sub> CO<sub>2</sub>, H<sub>2</sub>;
- e) incubating the step (d) mixture at 90°;
- f) obtaining the seed population of multibacterial strain;
- g) preparing nutrient medium II by adapting steps (a) to (c);
- h) inoculating the seed multibacterial strain under aseptic condition to step (g) nutrient medium II by maintaining impeller speed in the range of 30 to 40 r.p.m. anaerobic gas purging at the rate of 0.2 -0.5 vvm at a pH in the range of 7.0 to 7.50 and a at temperature in the range of 80°-90°C to obtain a biological solution;
- i) injecting the biological solution of step (h) to the oil well, followed by water to displace about 20% of entire biological solution, and
- j) obtaining the enhanced oil recovery from oil wells.

2. A process of claim 1, wherein in step (c) the nutrient medium I comprises of:

Mineral nutrients	Quantity/Litre
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5 to 1.5 g
K <sub>2</sub> HPO <sub>4</sub>	0.4 to 0.6 g
KH <sub>2</sub> PO <sub>4</sub>	0.1 to 0.6 g

<b>Nitrogenous substrates</b>	
<b>NH<sub>4</sub>Cl</b>	0.5 to 1.5 g
<b>Yeast extract</b>	1.0 to 4.0 g
<b>Tryptone</b>	0.5 to 1.0 g
<b>Reducing agents</b>	
<b>Cystein HCL</b>	1.0 to 5.0 g
<b>Na<sub>2</sub>S.9H<sub>2</sub>O</b>	2.0 to 5.0
<b>Carbon Source</b>	
<b>Molasses</b>	50.0 to 100 g
<b>Corn steep liquor</b>	50.0 to 100 g
<b>Buffering agent</b>	
<b>NaHCO<sub>3</sub></b>	1.0 to 2.5 g
<b>Vitamin stock solution</b>	10 to 20 ml
<b>Trace mineral stock solution</b>	15 to 20 ml

3. A process of claim 1, wherein in step (g) the nutrient medium II comprises of:

<b>Mineral nutrients</b>	<b>Quantity/Litre</b>
<b>MgSO<sub>4</sub>.7H<sub>2</sub>O</b>	0.05 to 0.15 g
<b>K<sub>2</sub>HPO<sub>4</sub></b>	0.2 to 0.4 g
<b>KH<sub>2</sub>PO<sub>4</sub></b>	0.2 to 0.4 g
<b>Nitrogenous substrates</b>	
<b>NH<sub>4</sub>Cl</b>	0.5 to 1.5 g
<b>Reducing agents</b>	
<b>Cystein HCL</b>	0.1 to 0.5 g
<b>Na<sub>2</sub>S.9H<sub>2</sub>O</b>	0.1 to 0.5 g
<b>Carbon Source</b>	
<b>Molasses</b>	15.0 to 30.0 g
<b>Corn steep liquor</b>	50-100 g
<b>Buffering agent</b>	
<b>NaHCO<sub>3</sub></b>	1.0 to 2.5 g
<b>Vitamin stock solution</b>	5 to 10 ml

Trace mineral stock solution	5 to 10 ml
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4. A process of claim 2, wherein the trace mineral solution in formation water having a pH in the range of 6.50 to 7.50 comprises of:-

Chemicals	Quantity per litre
Nitrilotriacetic acid (sodium salt)	0.82 to 2.00 g
MgSO <sub>4</sub>	2.5 to 3.2 g
MnSO <sub>4</sub> .2H <sub>2</sub> O	0.2 to 0.8 g
NaCl	0.7 to 1.2 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.05 to 0.12 g
CoCl <sub>2</sub> /CoSO <sub>4</sub>	0.07 to 0.14 g
ZnSO <sub>4</sub>	0.08 to 0.12 g
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.008 to 0.015 g
AlK (SO <sub>4</sub> ) <sub>2</sub>	0.007 to 0.015 g
H <sub>3</sub> BO <sub>3</sub>	0.009 to 0.012 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.008 to 0.13 g

5

- 5) A process of Claim 2, wherein the vitamin solution in distilled water having a pH in the range of 6.50 to 7.50 comprises of :

Vitamin	Quantity mg/l
Biotin	1.2 to 2.2
Folic acid	1.7 to 2.4
Pyridoxine HCl	7.0 to 12.0
Thamine HCl	7.0 to 12.0
Riboflavin	6.0 to 7.0
Nicotinic acid	4.0 to 6.0
DL-Calcium Pantothenate	3.0 to 6.2
P-Aminobenzoic acid	4.1 to 5.6
Vitamin B <sub>12</sub>	0.08 to 13.0
Lipoic acid	2.8 to 5.4

- 10 6) A process of claim 3, wherein the trace mineral solution in formation water having a pH in the range of 6.50 to 7.50 comprises of:-



Chemicals	Quantity per litre
Nitrilotriacetic acid (sodium salt)	0.82 to 2.00 g
MgSO <sub>4</sub>	2.5 to 3.2 g
MnSO <sub>4</sub> .2H <sub>2</sub> O	0.2 to 0.8 g
NaCl	0.7 to 1.2 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.05 to 0.12 g
CoCl <sub>2</sub> /CoSO <sub>4</sub>	0.07 to 0.14 g
ZnSO <sub>4</sub>	0.08 to 0.12 g
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.008 to 0.015 g
AlK (SO <sub>4</sub> ) <sub>2</sub>	0.007 to 0.015 g
H <sub>3</sub> BO <sub>3</sub>	0.009 to 0.012 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.008 to 0.13 g

7. A process of Claim 3, wherein the vitamin solution in distilled water having a pH in the range of 6.50 to 7.50 comprises of :

5

Vitamin	Quantity mg/l
Biotin	1.2 to 2.2
Folic acid	1.7 to 2.4
Pyridoxine HCl	7.0 to 12.0
Thamine HCl	7.0 to 12
Riboflavin	6.0 to 7.0
Nicotinic acid	4.0 to 6.0
DL-Calcium Pantothenate	3.0 to 6.2
P-Aminobenzoic acid	4.1 to 5.6
Vitamin B <sub>12</sub>	0.08 to 13.0
Lipoic acid	2.8 to 5.4

8. A process of claim 1, wherein in step (c) the alkali used is selected from a group consisting of sodium hydroxide and potassium hydroxide.

9. A process of claim 1, wherein nutrient media I and II are suitable for growing multibacterial strain consisting of Thermoanaerobacterium sp., Thermotoga sp. and Thermococcus sp.
10. A process of claim 9, wherein multibacterial strain obtained possess barophylic, thermophilic and anaerobic properties.
11. A process of claim 1, wherein nutrient medium II provides growth and proliferation of multi bacterial strain up to a temperature of 90°C in the oil well thereby enhancing the oil recovery.
12. A process of claim 1, wherein nutrient medium II helps in the growth of multi bacteria strain leading to the production of volatile fatty acids and carbon dioxide in the oil wells to enhance the recovery of oil.
13. A process of claim 12, wherein the volatile fatty acid produced reduces the viscosity of the oil in the oil well.
14. A process of claim 12, wherein the carbon-dioxide produced helps in sweeping the oil from the oil well.
15. A process of claim 1, wherein the oil recovery is enhanced up to 3 folds.
16. A process for enhancing the oil recovery from oil from oil well as herein described with reference to the examples.

Dated this 14 day of July 2003



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TO  
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**Abstract**

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The present invention provides a process for enhancing the oil recovery from oil wells, the said process comprising steps of: dissolving a mixture of mineral nutrients, reducing agents, buffering agent, carbon source and trace mineral in formation water, adjusting the pH, autoclaving, maintaining the temperature up to 90°C, adding vitamin solution, adjusting the pH; inoculating with multibacterial strain deposited with Institute of Microbial Technology, Chandigarh having accession no MTCC:S2-001; in the presence of an anaerobic gas mixture of  $N_2$   $CO_2$ ,  $H_2$ ; incubating at 90°C to obtain seed population of multibacterial strain; inoculating the seed multibacterial strain under aseptic condition on to a nutrient medium II to obtain a biological solution; injecting the biological solution to the oil well, followed by water to displace about 20% of entire biological solution and obtaining the enhanced oil recovery from oil wells

DUPLICATE